REMARKS

Entry of this amendment with allowance of the application is requested.

Non-elected claims 18-26 have been canceled without prejudice to divisional filing. This leaves claims 1-9 and 11-17 for consideration. These claims are thought to be allowable for the reasons noted below. Accordingly, favorable reconsideration is requested.

The Examiner has rejected claims 1, 2, 4, 5, 8, 9 and 11 under Section 102(b) as anticipated by the newly cited Sakai et al. reference (U.S. 5,445,042). Claims 1-9 and 11 have also been rejected under Section 103(a) as unpatentable over Sakai et al. and claims 1-9 and 11-17 have been rejected under Section 103(a) as unpatentable over Sakai et al. in view of newly cited Green (WO 01/28600). The Examiner has made these rejections final notwithstanding the citation of completely new art to reject the claims. The applicants request the Examiner to withdraw the finality of the rejection in view of the citation of completely new art to reject the claims. The Examiner states that the applicants' amendments necessitated the citation of the new art. With respect, however, it is submitted that the art, if considered relevant, could have been earlier cited as the nature of the applicants' invention was evident from the claims as originally presented.

In any case, the applicants respectfully request reconsideration of the Examiner's Section 102(b) and 103(a) rejections. With respect, it is submitted that the applicants' invention is both novel over Sakai et al. and unobvious therefrom considered with or without the Green reference.

The Examiner's rejections are based on at least two fundamental errors, i.e. the view that the "process" language in the applicants' claims should be disregarded as not adding patentability and, further, that the Sakai compositions are sterile because they include an alcohol component. The applicants submit that the "process" terminology used in the applicants' claims is properly definitive in the context of the applicants' invention and, therefore, clearly and unequivocally distinguishes over Sakai et al. Furthermore, Sakai's compositions are not sterilized as the applicants require. Alcohols may disinfect but this is not sterilization.

More specifically, the applicants submit that the Examiner must consider the applicants' claim language as properly and meaningfully descriptive of the claimed composition. In particular, the requirement that the applicants' composition "has been subjected to sterilizing radiation" and that the ion sources are used in amount "sufficient to maintain activity of the enzyme after radiation sterilization" are properly and distinctively definitive of the applicants' sterilized enzyme composition. Those in

the art know that radiation is an aggressive process that has distinctive effects on a composition, particularly where enzymes are involved. Accordingly, the "process" language which the applicants have used is necessarily descriptive of their composition and not simply an artificial way of distinguishing from an otherwise old material. Sakai clearly makes no mention whatsoever of sterilizing an enzyme composition by radiation. This alone should be enough to distinguish the applicants' claims from Sakai. Furthermore, as indicated above and detailed below, Sakai's compositions are not sterile. Hence, even if one disregards the radiation characteristic of the applicants' compositions, the claimed compositions are not disclosed by Sakai.

In connection with the foregoing, the applicants wish to emphasize that not all "process" limitations in a composition claim can be dismissed as inconsequential in determining patentability. For example, a "hydrated" product is one that has been hydrated. This has process connotations but the language is also descriptive of the composition and defines the nature of the composition, i.e. it includes water and, therefore, distinguishes over a composition that has no water. Similarly, a reference to "concentrated" has "process" connotations but it is also properly descriptive of a characteristic of a product. The same is true in the present case where the product is defined as having been subjected to sterilizing irradiation. This is, in a sense, a process description but those in the art would know from the language used that the composition is different as a composition because it has been subjected to sterilizing radiation. Sterilizing radiation is particularly aggressive and especially damaging to enzymes with property effects which are characteristic results of the radiation. Thus, in essence, the reference in the applicants' claims to sterilization by radiation is a compositional description to one in the art.

What the MPEP contemplates in its reference to an improper product-by-process claim is a claim which recites the process by which it is made but is the same as the products made using a different process. Clearly, rejection of a claim where the product is the same but is made in a different way is appropriate. This is not the situation here where the process referred to defines a characteristic of the claimed composition, i.e. it has been irradiated by sterilizing irradiation. There is no other way to describe this feature of the applicants' compositions. The applicants' language should, therefore, be acceptable to distinguish over the Sakai et al. compositions. How else would the Examiner define a product that has been subjected to sterilizing irradiation? Such a product is clearly different from one that has not been subjected to such irradiation. As noted, the purpose of the MPEP

position on "product-by-process" is to preclude argument as to patentable difference when the same product is made by a different process. This is not the case here because the "process" features result in a different composition

It stands admitted that Sakai et al. does not disclose an enzyme preparation that has been sterilized by irradiation. This alone should be enough to distinguish the applicants' claims from Sakai et al. under Section 102(b). Furthermore, taking into account the aggressive nature of irradiation and its impact on enzyme activity, the applicants' provision of compositions which mainain enzyme activity despite being sterilized by irradiation should also be considered unobvious from Sakai on this basis.

Furthermore, regardless of the Examiner's position on the product-by-process aspects of the applicants' claims, it is submitted that the applicants' claims differ from Sakai et al. in the requirement that the composition be sterilized. The Examiner considers that the compositions disclosed in Sakai et al. are sterile, in view of the fact that they comprise ethanol and isopropanol. However, the applicants respectfully disagree with this conclusion. Thus, despite the comments in Sakai et al. regarding sterility, it is simply not the case that including alcohols in a formulation can provide a sterile composition. In this regard, the applicants attach an extract from the textbook "Mims' Medical Microbiology, 4th Edition, which clearly states on page 568 that "sterilization is the process of killing or removing all viable organisms", and that "disinfection is a process of removing or killing most, but not all, viable organisms". On page 569, it can be seen that sterilization may be achieved by irradiation and page 570, it states that the process is continuous and one hundred percent efficient.

Reinforcing what is said in the introduction to the applicants' specification, Mims also states that irradiation can cause materials to deteriorate and thus is not suitable for resterilization of equipment. Page 571 states that there are many different antimicrobial chemicals available, but few are sterilant. Alcohols are listed in Table 36.6 as an example of disinfectants for use in hospitals, and it is notable that they are not mentioned as being able to provide sterility.

Also attached is an extract from "Microbiology, Principals and Explorations", 7th Edition. On page 342, it is stated that "sterilization is killing or removal of all micro-organisms in a material or on an object. There are no degrees of sterility – sterility means that there are no living organisms in or on a material." It is also stated that "disinfection means reducing the number of pathogenic organisms on objects or materials so that they pose no threat of disease". Page 343 discusses the effect of ethyl and isopropyl alcohols, and states that such materials can disinfect, however

they are not mentioned as being able to provide sterility. Alcohols are again discussed on page 349, where it is stated that "alcohol disinfects but does not sterilize skin because it evaporates quickly and stays in contact with microbes for only a few seconds". This reference also states that "it kills vegetative micro-organisms on the skin surface but does not kill endospores, resistant cells, or cells deep in skin pores."

In other words, alcohols do not provide sterility. Again, Table 12.3 on page 352 lists alcohols for use in disinfecting skin and no mention of sterility is present. On the contrary, page 358 lists ionizing radiation as being suitable for sterilizing medical equipment

Thus, in short, the applicants' claims distinguish over Sakai et al. not only in calling for a composition that has been subjected to sterilization by irradiation, which Sakai et al. does not disclose, but also in requiring sterilization which cannot be achieved by the use of alcohol as disclosed by Sakai et al.

In view of the foregoing, the applicants submit that their claims are directed to subject matter which is novel and patentable over Sakai et al. Clearly, it is not obvious from Sakai et al. how to provide a sterile enzyme composition of any kind, much less one made sterile by irradiation. Those in the art know that sterilization by irradiation is a particularly aggressive process which is especially damaging to enzymes. The applicants have found a way to sterilize an enzyme composition while maintaining enzyme activity by providing compositions as claimed. There is no suggestion in Sakai et al. that an enzyme preparation which has been subjected to irradiation could maintain its activity by including lactate ion and a source of zinc ions and/or a source of ammonium ions, as called for by the applicants. The use of radiation in the presence of enzymes is generally considered to be highly damaging. The present invention overcomes this concern in the art, by use of suitable materials, i.e. lactate ions and a source of zinc ions and/or a source of ammonium ions, which has been found by the present inventors to protect the enzyme from the damaging effects of radiation. There is nothing in Sakai et al. suggestive of this nor could the applicants' results be inherent in the practice of the Sakai et al. disclosure.

For the reasons noted, it is submitted that the Examiner's Section 102(b) and Section 103(a) rejections based on Sakai et al. should be withdrawn. Furthermore, Green does not fill in the fundamental deficiencies in Sakai et al. as noted above. Accordingly, the applicants submit hat the Section 103(a) rejection based on the combination of Sakai et al. and Green should also be withdrawn.

In summary, the applicants' claims are thought to be patentable over the art relied on by the Examiner. Accordingly, favorable reconsideration, with allowance, is requested.

Respectfully submitted,

MORGAN LEWIS & BOCKIUS LLP

By _____
Paul N. Kokulis
Reg. No. 16773

Date: February 3, 2009

Customer No. 09629
1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
Phone: (202) 739-3000
Facsimile: (202) 739-3001

Mims' Medical Microbiology

4th Edition

Richard V Goering BA MSc PhD

Professor and Chair
Department of Medical Microbiology and Immunology
Creighton University Medical Center
School of Medicine
Omaha, Nebraska USA

Hazel M Dockrell BA (Mod) PhD

Professor of Immunology
Department of Infectious and Tropical Diseases
London School of Hygiene & Tropical Medicine
London, UK

Derek Wakelin BSc PhD DSc FRCPath

Emeritus Professor, School of Biology
University of Nottingham
Nottingham, UK

Mark Zuckerman BSc (Hons) MB BS MRCP MSc FRCPath

Consultant Virologist and Honorary Senior Lecturer
South London Specialist Virology Centre and Health Protection Agency, London
King's College Hospital NHS Foundation Trust
Guy's, King's and St Thomas' School of Medicine
London, UK

Peter L Chiodini BSc MBBS PhD FRCP FRCPath FFTM RCPS (Glas)

Consultant Parasitologist
Hospital for Tropical Diseases
London, UK

Ivan M Roitt DSc HonFRCP FRCPath FRS

Emeritus Professor of Immunology
Department of Immunology and Molecular Pathology
Windeyer Institute of Medical Sciences
London, UK

Cedric Mims BSc MD FRCPath

Emeritus Professor
Department of Microbiology
Guy's Hospital Medical School
London, UK

MOSBY

ELSEVIER

MOSBY

An imprint of Elsevier Limited

First edition published by Mosby-Year Book Europe Ltd, 1993
Second edition published by Mosby-Year Book Europe Ltd, 1998
Third edition published 2004

Main Edition ISBN: 9780323044752
International Edition ISBN: 9780808923725

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging in Publication Data
A catalog record for this book is available from the Library of Congress

Notice
Medical knowledge is constantly changing. Standard safety precautions must be followed, but as new research and clinical experience broaden our knowledge, changes in treatment and drug therapy may become necessary or appropriate. Readers are advised to check the most current product information provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and contraindications. It is the responsibility of the practitioner, relying on experience and knowledge of the patient, to determine dosages and the best treatment for each individual patient. Neither the Publisher nor the authors assume any liability for any injury and/or damage to persons or property arising from this publication.

The Publisher

Printed in China
Last digit is the print number: 9 8 7 6 5 4 3 2 1

a hospital setting. The use of personal protective equipment (PPE) that included an N95 respirator, eye protection, mask, gloves and gown was mandatory to reduce the chance of transmission. Disposable second layers of clothing were also used, for example outer gloves, a gown and hand and foot covering.

STERILIZATION AND DISINFECTION

It is clear that the prevention of hospital infection depends in part upon the availability of clean, and where necessary, sterile equipment, instruments and dressings, isolation facilities and the safe disposal of infected material. Sterilization and disinfection are often talked about by microbiologists in relation to the production of sterile culture media and other laboratory activities, but it must be stressed that the concept of sterility is central to almost all areas of medical practice. An understanding of the rationale of sterilization and disinfection will aid intelligent use of the range of sterile equipment (from needles to prostheses) and techniques (from surgery to handwashing) employed in medical practice.

Definitions

Sterilization is the process of killing or removing all viable organisms

An item that is sterile is free from all viable organisms – in this sense, viable means capable of reproducing. Sterilization is achieved by physical or chemical means, either by the removal of organisms from an object or by killing the organisms *in situ*, sometimes leaving toxic breakdown products (pyrogens) in the object.

Disinfection is a process of removing or killing most, but not all, viable organisms

Disinfection employs either:

- a chemical 'disinfectant', which kills pathogens but may not kill viruses or spores
- a physical process such as boiling water or low pressure steam, which reduces the bioburden (i.e. the load of viable organisms).

Antiseptics are used to reduce the number of viable organisms on the skin

Antiseptics are a particular group of disinfectants. Some act differentially, destroying the transient flora but leaving the normal skin flora deep in the skin pores and hair follicles untouched (Fig. 36.13). It is impossible to sterilize the skin, but thorough washing with antiseptic soaps can reduce the numbers of organisms on the surface considerably and therefore reduce contact spread of infection (see above). However, the resident bacteria in the hair follicles and ducts of sweat glands can recolonize the skin surface within hours.

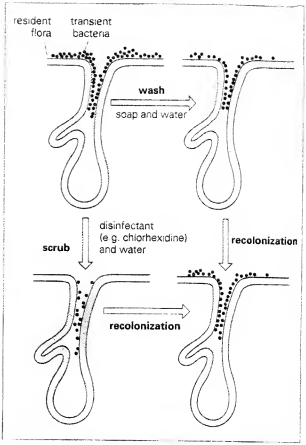


Figure 36.13 Normal skin is colonized with bacteria both on the surface and deep in the pores and ducts of the sweat and sebaceous glands. In addition, bacteria may be carried transiently on the skin surface and may be transmitted from a contaminated source to a susceptible patient. Careful handwashing with soap and water removes the transient flora and some of the superficial resident flora. Scrubbing the hands with disinfectants removes more of the resident flora, but the skin surface is recolonized within hours from the normal flora deep in the skin pores.

Pasteurization can be used to eliminate pathogens in heat-sensitive products

Pasteurization reduces the total numbers of viable microbes in bulk fluids such as milk and fruit juices without destroying flavor and palatability. It does not affect spores, but is effective against intracellular organisms such as *Brucella* and mycobacteria and many viruses.

Since the beginning of recorded history, various other techniques have been used to prevent the multiplication of microorganisms, such as drying and salting of food.

Deciding whether sterilization or disinfection should be used

Sterilization and disinfection processes are costly, and so it is important to choose the appropriate method and the one that causes the least damage to the material involved. A variety of considerations influence the choice of method. The detailed mechanisms of the death process of micro-

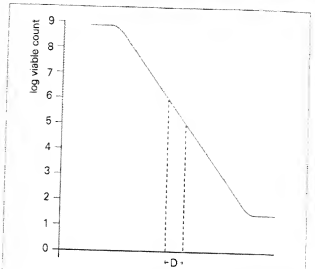
organisms may vary with the sterilizing technique used, but the net effect is similar in that essential cell constituents (nucleic acids or proteins) are inactivated.

It is easier to sterilize a clean object than a physically dirty one

This is because organic matter protects microbes and hinders penetration of heat or chemicals and may inactivate certain chemicals. In other words, a low bioburden is a prerequisite for cost-effective sterilization.

The rate of killing of microorganisms depends upon the concentration of the killing agent and time of exposure

The number of organisms surviving sterilization can be expressed by the equation: N is proportional to $1/CT$, where N is the number of survivors, C is the concentration of agent and T is time of exposure to the agent. If a population of microbes is exposed to a sterilizing technique, and the number of survivors expressed as a logarithm is plotted against time, the slope of the graph defines the death rate (Fig. 36.14). These lines may be sigmoid or have shoulders, indicating that individual cells respond slightly differently, some being killed more easily than others. In the case of bacteria, the physiologic state of the organisms influences the shape of the killing curve; young, replicating cells are usually more vulnerable than stationary or decline-phase organisms or those that are sporing. Graphs like those shown in Figure 36.14 can be used to predict the conditions necessary to achieve sterility. However, these experimental data are usually based on pure cultures in the laboratory (bacterial spores are often used as model systems), whereas in real life, the bioburden is mixed. Therefore, predictions from such data may be inappropriate for mixed populations.



D = decimal reduction time (i.e. time required to reduce the population by 90% at a specified temperature)

Bacillus cereus	$D_{121} = 2.4$ min
Bacillus stearothermophilus	$D_{122} = 3.4$ min
Clostridium botulinum	$D_{104} = 5.5$ min
Clostridium perfringens	$D_{104} = 2.3$ min

Figure 36.14 Theoretically, there is a straight line relationship between the log viable count of a bacterial population and time when the population is exposed to a lethal temperature. In practice these lines are usually sigmoid. The D value is the time required to reduce the population by 90% at a specified temperature. *Bacillus stearothermophilus* spores are used as biologic indicators of effective heat sterilization by including filter paper strips carrying a standard number of spores into the autoclave cycle. The strips are then incubated to attempt to recover viable organisms. The usual autoclave cycle of 121°C for 15 min is adequate to kill *B. stearothermophilus* with a margin of safety.

Techniques for sterilization

Sterilization may be achieved by:

- heat
- irradiation (gamma or ultraviolet)
- filtration
- chemicals in liquid or gaseous phase.

Other techniques of doubtful efficiency include freezing and thawing, lysis, dessication, ultrasonication and the use of electrical discharges, but these are not applied in hospital practice.

Ultraviolet irradiation is inefficient as a sterilant, and its important uses in the hospital setting are in inhibiting growth of bacteria in water in complex apparatus such as auto-analyzers and in air in safety hoods in virology laboratories. The potential for damage to the cornea and skin precludes wider use of ultraviolet irradiation. It should be remembered that the agents of Creutzfeldt–Jakob disease (CJD), bovine spongiform encephalopathy (BSE) and scrapie are highly resistant and are not completely inactivated by formalin, ultraviolet irradiation, ionizing radiation or regular autoclaving. Sterilization can be achieved by boiling in 1N NaOH for 10 min at atmospheric pressure followed by autoclaving at a higher than normal temperature for a longer period than usual (134°C for 18 min), but obviously this technique cannot be applied to living tissues or materials that are damaged at high temperatures.

Heat

Heat, as a way of transferring energy, is the preferred choice for sterilization on the grounds of ease of use, controllability, cost and efficiency.

Dry heat sterilizes by oxidation of the cell components

Incineration and the use of the laboratory Bunsen burner are examples of sterilization by dry heat. Glassware can be sterilized in a hot air oven at 160–180°C for 1 h.

The most effective agent for sterilization is saturated steam (moist heat) under pressure

This can be achieved using an autoclave. Steam under pressure aids penetration of heat into the material to be sterilized (such as dressings), and there is a direct relationship between temperature and steam pressure. Steam under pressure has a temperature in excess of 100°C, which results in increased killing of microbes.

Sterilizing efficiency is improved by evacuating all of the air from the autoclave chamber. The subsequent introduction of high pressure steam rapidly penetrates to all parts of the chamber and its load, and results in predictable rises in temperature in the center of articles to be sterilized. The length of an autoclave cycle is determined by the holding time plus a margin of safety, and is derived from the thermal death curves for heat-resistant pathogens such as clostridia. Therefore, the usual cycle of 121°C for 15 min is sufficient to kill the spores of *Cl. botulinum* with an adequate margin of safety. However, the spores of some bacterial species, especially soil organisms, are able to withstand this temperature. The safety margin is reduced in the presence of large numbers of organisms because there is a greater probability of more heat-resistant individuals existing in a large population; hence, the importance of cleaning instruments, whenever possible, before sterilization.

Moist heat in an autoclave is used to sterilize surgical instruments and dressings and heat-resistant pharmaceuticals. A method for the sterilization of heat-sensitive instruments such as endoscopes uses a combination of low temperature (subatmospheric) steam and formaldehyde.

All of these processes need to be carried out in a suitable pressure vessel and are therefore usually available in the hospital central sterile supply department.

Immersion in boiling water for a few minutes can be used as a rapid emergency measure to disinfect instruments

Immersion in boiling water for a few minutes will kill vegetative bacteria and many, but not all, spores. The addition of 2% sodium carbonate to the water potentiates the sporicidal effect.

Pasteurization uses heat at 62.8–65.6°C for 30 min

This technique was devised by Pasteur to prevent the spoilage of wine by heating it to 50–60°C. It is now used for fluids such as milk to reduce the number of bacteria. This helps to eliminate pathogens present in small numbers and to improve the shelf-life of milk. The fluid is held at a temperature of 62.8–65.6°C for 30 min or may be 'flash' pasteurized at 71.7°C for 15 s. After either process, the fluid should be kept at a temperature below 10°C to minimize subsequent bacterial growth.

Irradiation

Gamma irradiation energy is used to sterilize large batches of small volume items

The use of gamma irradiation energy for sterilization is an industrial process that works well with products such as needles, syringes, intravenous lines, catheters and gloves. It can also be used for vaccines and to prevent food spoilage. Although the capital cost of the equipment is high, the process is continuous and 100% efficient. Articles are sterilized while sealed in their original packaging, without any heat gain. The process must be conducted in a suitably constructed building, usually at a location distinct from the hospital and usually outside the hospital administration. However, irradiation can cause materials to deteriorate and is thus not suitable for resterilization of equipment. The killing mechanism involves the production of free radicals, which break the bonds in DNA. Irradiation kills spores, but at a higher dose than vegetative cells because of the relative lack of water in spores.

Sterilization using ultraviolet irradiation is discussed above.

Filtration

Filters are used to produce particle- and pyrogen-free fluid

Solutions that are heat-sterilized will contain pyrogens. These heat-stable breakdown products of microbes are capable of inducing fever and are therefore undesirable in products such as intravenous fluids. Filtration or separation of the product from the contamination has a long history in the clarification of water and wine. Modern filters are composed of nitrocellulose and work by electrostatic attraction and physical pore size to retain organisms or other particles. The resulting fluid should be particle-free. Filtration is used in some parts of the world to purify drinking water.

Filtration techniques are also used to recover very small numbers of organisms from very large volumes of fluid (e.g. *Legionella* from cooling tower water) and can be used as a method for quantifying bacteria in fluids.

Chemical agents

The gases ethylene oxide and formaldehyde kill by damaging proteins and nucleic acids

The need for sterilization by gaseous chemicals has been greatly reduced by the success of gamma irradiation (see above), but two alkylating gases, ethylene oxide and formaldehyde, are still used:

- Ethylene oxide is used in some centers to sterilize single use medical requisites such as heart valves. However, it is toxic and potentially explosive
- Formaldehyde is not explosive, but has an extremely unpleasant odor and is an irritant to mucous membranes. It has been used as a disinfectant to

decontaminate rooms (such as isolation rooms) and in the laboratory to disinfect exhaust-protective cabinets. A high relative humidity is essential for effective killing.

The liquid glutaraldehyde is used to disinfect heat-sensitive articles

Glutaraldehyde is less toxic than formaldehyde and can be stabilized in solution to remain active for up to several weeks at in-use concentration. It is used for the disinfection of, but does not sterilize, heat-sensitive articles such as endoscopes and for inanimate surfaces.

Many different antimicrobial chemicals are available, but few are sterilant

Some, like the derivatives of pine and turpentine, have been known since ancient times, and chloride of lime and coal tar fluids were in use before the germ theory of disease was established. Most fall into the category of disinfectant or antiseptic, but a few are capable of rendering articles sterile. Factors that affect their efficacy include:

- physical environment (e.g. porous or cracked surfaces)
- presence of moisture
- temperature and pH
- concentration of the agent
- hardness of water
- the bioburden on the object to be disinfected
- the nature and state of the microbes in the bioburden
- the ability of the microbes to inactivate the chemical agent.

It is obvious that the above factors are difficult to control in every circumstance. The main groups of chemical agents are shown in Table 36.6. They act by causing chemical damage to proteins, nucleic acids or cell membrane lipids. The activity of a given disinfectant may result from more than one pathway of damage.

Table 36.6 Examples of disinfectants for use in hospitals

Group	Examples	Advantages and disadvantages
Phenolics	Clear soluble phenolic compounds, white fluids	Good general-purpose disinfectants, not readily inactivated by organic matter, active against wide range of organisms including *mycobacteria*, not sporicidal
	Chloroxylenols	Inactivated by hard water and organic matter, *Pseudomonas* grows readily in chloroxylenol solutions, limited activity against other Gram-negatives
Halogens	Hypochlorites (chloramine)	Cheap, effective, act by release of free chlorine, active against viruses and therefore recommended for disinfection of equipment soiled with blood (because of hepatitis and HIV risk), inactivated by organic material, corrode metals
	Iodine and iodophors	Useful skin disinfectants, sporicidal
Quaternary ammonium compounds	Benzalkonium chloride, cetyltrimethylammonium bromide	Have detergent properties, activity against Gram-negative ← Gram-positive, improved by combination with biguanide, e.g. chlorhexidine, useful as skin disinfectants, inactivated by hard water and organic materials, contamination of stock solutions with Gram-negative rods can be a problem
Diguanides	Chlorhexidine	Useful disinfectant for skin and mucous membranes, inactivated by many materials and too expensive for environmental use, alcoholic solutions are less easily contaminated, combinations of chlorhexidine and detergent highly effective for disinfection of hands
Alcohols	Ethyl alcohol, isopropyl alcohol	Good choice for skin disinfection and for clean surfaces, sometimes used in combination with iodine or chlorhexidine (see above), water must be present for bacterial killing (i.e. 70% ethanol best), isopropyl preferred for skin and articles in contact with patient
Aldehydes	Formaldehyde/formalin	Too irritant for use as general disinfectant
	Glutaraldehyde	Kills vegetative organisms, including mycobacteria, slowly but effectively more active, less toxic than formaldehyde, sporicidal (within 6 h when fresh), slightly irritant, used in alkaline solution which is stable for 1–2 weeks, expensive, limited use, e.g. disinfection of endoscopes
Chlorinated bisphenols	Hexachorophene	Activity against Gram-positive → Gram-negative, used in soap or dusting powder as skin disinfectant (use restricted after potentially toxic blood levels found in infants who had hexachlorophene emulsion spread over whole body)
		Introduced as substitute for hexachlorophene in soap, considerable antibacterial effect on repeated use

Note that no one disinfectant has all the properties desirable for use both on skin and on inanimate surfaces

International Student Version

MICROBIOLOGY

PRINCIPLES AND EXPLORATIONS

JACQUELYN G. BLACK

Marymount University, Arlington, Virginia

Contributor: **LAURA J. BLACK**

Laura Black has been working on this book since she was ten years old. She has now been brought on as a contributing author for the seventh edition.

Jacquelyn and Laura Black

WILEY JOHN WILEY & SONS,

To Laura...
for sharing her mother and much of her childhood
with that greedy sibling "the book."

Senior Acquisitions Editor Kevin Witt
Associate Editor Merillat Staat
Senior Production Editor Elizabeth Swain
Executive Marketing Manager Clay Stone
Text Designer Madelyn Lesure
Cover Designers Madelyn Lesure and Merillat Staat
Senior Illustration Editor Anna Melhorn
Photo Editor Hilary Newman
Photo Researcher Mary Ann Price
Senior Media Editor Linda Muriello
Editorial Assistant Alissa Rufino
Associate Director of Education Belinda Tan
Production Executive Jessie Yeo
Senior Marketing Manager Angela Teo
ISV Project Editor Gladys Soto
ISV Production Manager Micheline Frederick
ISV Cover Designer Michael St. Martine

ISV ISBN: 978-0470-23415-0
Printed in Asia
10 9 8 7 6 5 4 3 2 1

Do you like spicy foods? Perhaps you won't like the original reasons for their popularity. Before modern methods of food preservation, such as canning and refrigeration, were available, control of microbial growth in foods was a difficult problem. Inevitably after a short while, food began to take on the "off" flavors of spoilage. Spices were used to mask these unpleasant tastes. Some spices were also effective as preservatives. The antimicrobial effects of garlic have long been known. Fortunately, we need not eat spoiled food today, and we can use spices solely to enhance our enjoyment of safely preserved foods.

Medical care, especially in the operating room, is also safer today. As we have seen from the work of Ignaz Semmelweis and Joseph Lister, careful washing and the use of chemical agents are effective in controlling many infectious microorganisms (◀Chapter 1, p. 14). In this chapter, we will consider the properties of various chemical and physical agents used to control microorganisms in laboratories, in medical facilities, and in homes. Go to the website for this chapter to read about the specialized career of hospital infection control practitioner. This is a board-certified career open to both nurses and biology majors. The web essay also details specific methods of sterilization and disinfection used in hospitals.

PRINCIPLES OF STERILIZATION AND DISINFECTION

Sterilization is the killing or removal of all microorganisms in a material or on an object. There are no degrees of sterility—**sterility** means that there are *no* living organisms in or on a material. When properly carried out, sterilization procedures ensure that even t resistant bacterial endospores and fungal spores are k Much of the controversy regarding spontaneous ge tion in the nineteenth century resulted from the fail kill resistant cells in materials that were thought to be ile. In contrast with sterilization, **disinfection** means cing the number of pathogenic organisms on objects materials so that they pose no threat of disease.

Agents called **disinfectants** are typically applie inanimate objects, and agents called **antiseptics** ar plied to living tissue. A few agents are suitable as disinfectants and antiseptics, although most disinfec are too harsh for use on delicate skin tissue. *Antih* though often applied to skin, are considered separ in ◀Chapter 13. Terms related to sterilization and d fection are defined in **Table 12.1**.

BIOTECHNOLOGY

Microbes in Space

Never a problem for Han Solo, but in the real world of space travel, bacteria carried by astronauts are of real concern. Some of the problems include infectious diseases, allergy to microbial metabolites, and microbial deterioration of structural materials. Preventing microbial problems inside space vehicles requires limiting the routes of infectious disease and the use of an efficient wastewater recovery system. Although sterility is not possible, most of the familiar routes of disease transmission are present within space vehicles: water, food, aerosols, and environmental surfaces. Providing potable water for extended space travel has been a challenge from the very beginning of the space program. For the past 15 years various prototype wastewater recovery systems have been under development at NASA's (National Aeronautics and Space Administration) Marshall Space Flight Center. Using these systems, potable water is produced when humidity condensate, hygiene water, urine, and fuel cell water are collected and treated to remove microbes and chemical contaminants.

THE CONTROL OF MICROBIAL GROWTH

As explained in the discussion of the growth curv ◀Chapter 6 (p. 149), both the growth and death of m organisms occur at logarithmic rates. Here we are conce with the death rate and the effects on it of antimicr agents—substances that kill microbes or inhibit their gr

Organisms treated with antimicrobial agents the same laws regarding death rates as those declini numbers from natural causes. We will illustrate this ciple with heat as the agent because its effects have the most thoroughly studied. When heat is applied material, the death rate of the organisms in or remains logarithmic but is greatly accelerated. Heat as an antimicrobial agent. If 20% of the organisms d the first minute, 20% of those remaining alive wil in the second minute, and so on. If, at a different temp ture, 30% die in the first minute, 30% of the remai ones will die in the second minute, and so on. From t observations we can derive the principle that *a de proportion of the organisms die in a given time interva*

Consider now what happens when the number of organisms that remain becomes small—100, for exan At a death rate of 30% per minute, 70 will remain 1 minute, 49 after 2 minutes, 34 after 3 minutes, and 1 after 12 minutes. Soon the probability of finding ev single live organism becomes very small. Most lab tories say a sample is sterile if the probability is no gre than one chance in a million of finding a live organism

The total number of organisms present when d fection is begun affects the length of time require eliminate them. We can state a second principle: *fewer organisms present, the shorter the time needed achieve sterility*. Thoroughly cleaning objects be attempting to sterilize them is a practical applicatio this principle. Clearing objects of tissue debris and bi is also important because such organic matter imp the effectiveness of many chemical agents.

Different antimicrobial agents affect various speci bacteria and their endospores differently. Furthermore,

TABLE 12.1

Terms Related to Sterilization and Disinfection

Term	Definition
Sterilization	The killing or removal of all microorganisms in a material or on an object.
Disinfection	The reduction of the number of pathogenic microorganisms to the point where they pose no danger of disease.
Antiseptic	A chemical agent that can safely be used externally on living tissue to destroy microorganisms or to inhibit their growth.
Disinfectant	A chemical agent used on inanimate objects to destroy microorganisms. Most disinfectants do not kill spores.
Sanitizer	A chemical agent typically used on food-handling equipment and eating utensils to reduce bacterial numbers so as to meet public health standards. Sanitization may simply refer to thorough washing with only soap or detergent.
Bacteriostatic agent	An agent that inhibits the growth of bacteria.
Germicide	An agent capable of killing microbes rapidly; some such agents effectively kill certain microorganisms but only inhibit the growth of others.
Bactericide	An agent that kills bacteria. Most such agents do not kill spores.
Viricide	An agent that inactivates viruses.
Fungicide	An agent that kills fungi.
Sporocide	An agent that kills bacterial endospores or fungal spores.

given species may be more susceptible to an antimicrobial agent at one phase of growth than at another. The most susceptible phase for most organisms is the logarithmic growth phase, because during that phase many enzymes are actively carrying out synthetic reactions, and interfering with even a single enzyme might kill the organism. From these observations, we can state a third principle: *Microorganisms differ in their susceptibility to antimicrobial agents.*

CHEMICAL ANTIMICROBIAL AGENTS

THE POTENCY OF CHEMICAL AGENTS

The potency, or effectiveness, of a chemical antimicrobial agent is affected by time, temperature, pH, and concentration. The death rate of organisms is affected by the length of time the organisms are exposed to the antimicrobial agent, as was explained earlier for heat. Thus, adequate time should always be allowed for an agent to kill the maximum number of organisms. The death rate of organisms subjected to a chemical agent is accelerated by increasing the temperature. Increasing temperature by 10°C roughly doubles the rate of chemical reactions and thereby increases the potency of the chemical agent. Acidic or alkaline pH can increase or decrease the agent's potency. A pH that increases the degree of ionization of a chemical agent often increases its ability to penetrate a cell. Such a pH also can alter the contents of the cell itself. Finally, increasing concentration may increase the effects of most antimicrobial chemical agents. High concentrations may be **bactericidal** (killing), whereas lower concentrations may be **bacteriostatic** (growth inhibiting).

Both ethyl and isopropyl alcohol are exceptions to the rule about increasing concentrations. They have long been believed to be more potent at 70% than at higher concentrations, although they are also effective at up to 99% concentration. Some water must be present for alcohols to disinfect because they act by coagulating (permanently denaturing) proteins, and water is needed for the coagulation reactions. Also, a 70% alcohol-water mixture penetrates more deeply than pure alcohol into most materials to be disinfected.

EVALUATING THE EFFECTIVENESS OF CHEMICAL AGENTS

Many factors affect the potency of chemical antimicrobial agents, so evaluation of effectiveness is difficult. No entirely satisfactory method is available. However, we need some way to compare the effectiveness of disinfecting agents, especially as new ones come on the market. Should you believe the salesman when he tells you his is better? Ask him what its phenol coefficients are.

The Phenol Coefficient

Since Lister introduced *phenol* (carbolic acid) as a disinfectant in 1867, it has been the standard disinfectant to which other disinfectants are compared under the same conditions. The result of this comparison is called the **phenol coefficient**. Two organisms, *Salmonella typhi*, a pathogen of the digestive system, and *Staphylococcus aureus*, a common wound pathogen, are typically used to determine phenol coefficients. A disinfectant with a phenol coefficient of 1.0 has the same effectiveness as phenol. A coefficient less than 1.0 means that the disinfectant is less effective than phenol; a coefficient greater than 1.0 means that it is more effective. Phenol coefficients are reported separately for the different test organisms (**Table 12.2**). Lysol, for instance, has a coefficient of 5.0

TABLE 12.2

Phenol Coefficients of Various Chemical Agents

Chemical Agent	*Staphylococcus aureus*	*Salmonella typhi*
Phenol	1.0	1.0
Chloramine	133.0	100.0
Cresols	2.3	2.3
Ethyl alcohol	6.3	6.3
Formalin	0.3	0.7
Hydrogen peroxide	—	0.01
Lysol	5.0	3.2
Mercury chloride	100.0	143.0
Tincture of iodine	6.3	5.8

against *Staphylococcus aureus* but only 3.2 when used on *Salmonella typhi*, whereas ethyl alcohol has a value of 6.3 against both.

The phenol coefficient can be determined by the following steps. Prepare several dilutions of a chemical agent, and place the same volume of each in different test tubes. Prepare an identical set of test tubes, using phenol dilutions. Put both sets of tubes in a 20° C water bath for at least 5 minutes to ensure that the contents of all tubes are at the same temperature. Transfer 0.5 ml of a culture of a standard test organism to each tube. After 5, 10, and 15 minutes, use a sterile loop to transfer a specific volume of liquid from each tube into a separate tube of nutrient broth, and incubate the tubes. After 48 hours, check cultures for cloudiness, and find the smallest concentration (highest dilution) of the agent that killed all organisms in 10 minutes but not in 5 minutes. Find the ratio of this dilution to the dilution of phenol that has the same effect. For example, if a 1:1000 dilution of a chemical agent has the same effect as a 1:100 dilution of phenol, the phenol coefficient of that agent is 10 (1,000/100). If you performed this test on a new disinfectant and obtained these results, you would have found a very good disinfectant! The phenol coefficient provides an acceptable means of evaluating the effectiveness of chemical agents derived from phenol, but it is less acceptable for other agents. Another problem is that the materials on or in which organisms are found may affect the usefulness of a chemical agent by complexing with it or inactivating it. These effects are not reflected in the phenol coefficient number.

The Filter Paper Method

The **filter paper method** of evaluating a chemical agent is simpler than determining a phenol coefficient. It uses small filter paper disks, each soaked with a different chemical agent. The disks are placed on the surface of an agar plate that has been inoculated with a test organism. A different plate is used for each test organism. After incubation, a chemical agent that inhibits growth of a test organism is identified by a clear area around the disk where the bacteria have been killed (**Figure 12.1**). Note: What fective against one organism may have little or no effe the others. Will the chemical agent having the widest of inhibition around it be the most effective to use? It not be. Organic matter such as blood, feces, or vomitu interfere with its action. Also, some chemical agents just have molecules that are able to travel faster or fa through agar than the other agents tested did.

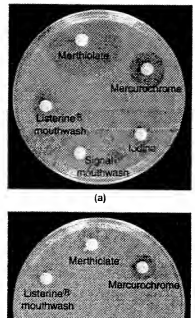


Figure 12.1 The paper method of evaluating disinfectants and antiseptics. The difference in response (a) *Staphylococcus au* (Gram-positive) (Jack Bostrack/Visuals Unlin and (b) *Escherichia co* (Gram-negative) to sev common chemical ag shown here. In both ca the greatest inhibition growth is seen near th of the Petri dish surrou the merthiolate-soake paper disk. The variou were soaked in mer curochrome, iodine, Si mouthwash, or Listeri mouthwash before be placed on the surface o nutrient medium, whic first been confluently inoculated with one of test organisms. (Jack M Bostrack/Visuals Unlim

The Use-Dilution Test

A third way of evaluating chemical agents, the **dilution test**, uses standard preparations of certain bacteria. A broth culture of one of these bacteria is ca onto small stainless steel cylinders and allowed to Each cylinder is then dipped into one of several dilu of the chemical agent for 10 minutes, removed, ri with water, and placed into a tube of broth. The tube incubated and then observed for the presence or abs of growth. Agents that prevent growth at the gre dilutions are considered the most effective. Many m biologists feel that this measurement is more meanin than the phenol coefficient.

DISINFECTANT SELECTION

Several qualities should be considered in deciding w disinfectant to use. An ideal disinfectant should

1. Be fast acting even in the presence of org substances, such as those in body fluids

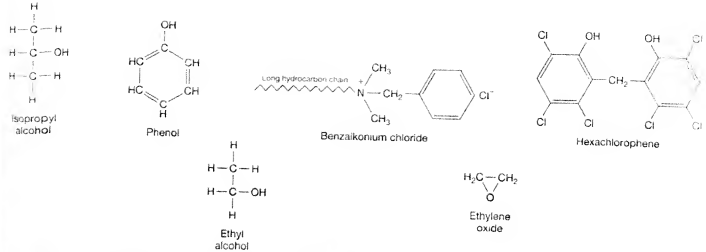


Figure 12.4 Structural formulas of some important disinfectants.

susceptible to inactivation when exposed to visible light. This process disrupts the structure of the viral nucleic acid.

Viruses sometimes remain infective even after their proteins are denatured, so methods used to rid materials of bacteria may not be as successful with infectious viruses. Also, use of an agent that does not inactivate viruses can lead to laboratory-acquired infections.

SPECIFIC CHEMICAL ANTIMICROBIAL AGENTS

Now that we have considered general principles of sterilization and disinfection and the kinds of reactions caused by such agents, we can look at some specific agents and their applications. The structural formulas of some of the most important compounds discussed are shown in **Figure 12.4**.

Soaps and Detergents

Soaps and detergents remove microbes, oily substances, and dirt. Mechanical scrubbing greatly enhances their action. In fact, vigorous hand washing is one of the easiest and cheapest means of preventing the spread of disease among patients in hospitals, in medical and dental offices, among employees and patrons in food establishments, and among family members. Unlike surgical scrubs, germicidal soaps usually are not significantly better disinfectants than ordinary soaps.

Soaps contain alkali and sodium and will kill many species of *Streptococcus*, *Micrococcus*, and *Neisseria* and will destroy influenza viruses. Many pathogens that survive washing with soap can be killed by a disinfectant applied after washing. A common practice after washing and rinsing hands and inanimate objects is to apply a 70% alcohol solution. Even these measures do not necessarily rid hands of all pathogens. Consequently, disposable gloves are used where there is a risk that health care workers may become infected or may transmit pathogens to other patients.

In public restrooms, only 68% of people are observed to wash their hands after using the toilet

Detergents, when used in weak concentrations in wash water, allow the water to penetrate into all crevices and cause dirt and microorganisms to be lifted out and washed away. Detergents are said to be *cationic* if they are positively charged and *anionic* if they are negatively charged. Cationic detergents are used to sanitize food utensils. Although not effective in killing endospores, they do inactivate some viruses. Anionic detergents are used for laundering clothes and as household cleaning agents. They are less effective sanitizing agents than cationic detergents, probably because the negative charges on bacterial cell walls repel them.

Some bacterial spores can survive 20 years sitting in 70% ethyl alcohol.

PUBLIC HEALTH

Soap and Sanitation

Washing and drying clothing in modern public laundry facilities is generally a safe practice because the clothing is almost disinfected if the water temperature is high enough. Soaps, detergents, and bleaches kill many bacteria and inactivate many viruses. Agitation of the clothes in the washer provides good mechanical scrubbing. Many microbes that survive this action are killed by heat in the dryer. The use of bar soap in a public washroom is not such a safe practice. The soap may be a source of infectious agents. In a study of 84 samples of bar soap taken from public washrooms, every sample contained microorganisms. More than 100 strains of bacteria and fungi were isolated from the soap samples, and some of the organisms were potential pathogens. Many restaurants and other establishments have installed soap dispensers for this very reason. In fact, many jurisdictions have made the use of bar soap in such facilities illegal.

TRY IT

How Well Do Those Waterless Hand Cleaners Work?

It's often difficult to find a place to wash your hands. Recently many products have appeared on the market, claiming to do a good job of cleaning your hands with a little gel instead of old-fashioned soap and water. But do they really work? Students in my labs have found that some are excellent, but many others have little or no effect. Here's a chance to plan a short research project of your own. Check with your instructor as to the validity of your design and methods, and of course, for permission to try this in lab.

Many cationic detergents are **quaternary ammonium compounds**, or **quats**, which have four organic groups attached to a nitrogen atom. The ammonium ion (NH_4) has four hydrogens, each of which can be replaced by an organic group binding to the central nitrogen atom. *Quat* is the abbreviation for the Latin *quattuor* meaning "four." A variety of quats are available as disinfecting agents; their chemical structures vary according to their organic groups. One problem with quats is that their effectiveness is decreased in the presence of soap, calcium or magnesium ions, or porous substances such as gauze. An even more serious problem with these agents is that they support the growth of some bacteria of the genus *Pseudomonas* rather than killing them. Zephiran (benzalkonium chloride) was once widely used as a skin antiseptic. It is no longer recommended because it is less effective than originally thought and is subject to the same problems as other quats. It is still very often found in "ear-piercing care" kits. Quats are now often mixed with another agent to overcome some of these problems and to increase their effectiveness. Zephiran dissolved in alcohol kills about twice as many microbes in the same time as does a water (aqueous) solution of the same amount of Zephiran. Mouthwashes that foam when shaken usually contain a quat.

Acids and Alkalis

Soap is a mild alkali, and its alkaline properties help destroy microbes. A number of organic acids lower the pH of materials sufficiently to inhibit fermentation. Several are used as food preservatives. Lactic and propionic acids retard mold growth in breads and other products. Benzoic acid and several of its derivatives are used to prevent fungal growth in soft drinks, ketchup, and margarine. Sorbic acid and sorbates are used to prevent fungal growth in cheeses and a variety of other foods. Boric acid, formerly used as an eyewash, is no longer recommended because of its toxicity.

Heavy Metals

Heavy metals used in chemical agents include selenium, mercury, copper, and silver. Even tiny quantities of such metals can be very effective in inhibiting bacterial growth (Figure 12.5). Silver nitrate was once widely used to

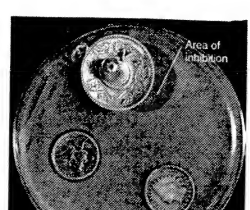


Figure 12.5 Heavy metals inhibit bacterial growth. The inhibitory effects of silver ions can be seen as clear zones in wh growth has occurred around the silver charm (which has been p aside) and silver dime. The nonsilver coin (a copper penny) has inhibited growth of the organisms as effectively as the silver ob have. *(Centers for Disease Control and Prevention CDC)*

prevent gonococcal infection in newborn infants. A drops of silver nitrate solution were placed in the i eyes at the time of delivery to protect against infecti gonococci entering the eyes during passage throug birth canal. For a time, many hospitals replaced silv trate with antibiotics such as erythromycin. Howeve development of antibiotic-resistant strains of gono has led some localities to require the use of silver ni to which gonococci do not develop resistance.

Organic mercury compounds, such as merthiolat mercurochrome, are used to disinfect surface skin wo Such agents kill most bacteria in the vegetative stat do not kill spores. They are not effective against *M bacterium*. Merthiolate is generally prepared as a tin (tingk'chur), that is, dissolved in alcohol. The alcoho tincture may have a greater germicidal action that heavy metal compound. Thimerosal, another organic cury compound, can be used to disinfect skin and ir ments and as a preservative for vaccines. Phenylmer nitrate and mercuric naphthenate inhibit both bacteria fungi and are used as laboratory disinfectants.

TRY IT

For a Clear Aquarium

If you have an aquarium, you probably have cor tended with water that looks like pea soup because of the l numbers of algae growing in it. This problem can be correct by placing a few pennies in the tank. Enough copper to inhib algal growth dissolves from the pennies into the water. For small investment, you can greatly increase visibility and enjo ment of your fish.

Selenium sulfide kills fungi, including spores. Preparations containing selenium are commonly used to treat fungal skin infections. Shampoos that contain selenium are effective in controlling dandruff. Dandruff, a crusting and flaking of the scalp, is often, though not always, caused by fungi. Mites sometimes play a role.

Copper sulfate is used to control algal growth. Although algal growth usually is not a direct medical problem, it is a problem in maintaining water quality in heating and air-conditioning systems and outdoor swimming pools. (The Environmental Protection Agency, however, is evaluating copper sulfate as an environmental hazard.)

Halogens

Hypochlorous acid, formed by the addition of chlorine to water, effectively controls microorganisms in drinking water and swimming pools. It is the active ingredient in household bleach and is used to disinfect food utensils and dairy equipment. It is effective in killing bacteria and inactivating many viruses. However, chlorine itself is easily inactivated by the presence of organic materials. That is why a substance such as copper sulfate is used to control algal growth in water to be purified with chlorine.

Iodine also is an effective antimicrobial agent. However, it should not be used on persons known to have an allergy to iodine. Often seafood allergies are triggered by iodine in the seafood. Tincture of iodine was one of the first skin antiseptics to come into use. Now *iodophors*, slow-release compounds in which the iodine is combined with organic molecules, are more commonly used. In such preparations, the organic molecules act as surfactants. Betadine and Isodine are used for surgical scrubs and on skin where an incision will be made. These compounds take several minutes to act and do not sterilize the skin. Betadine in concentrations of 3 to 5% destroys fungi, amoebas, and viruses, as well as most bacteria, but it does not destroy bacterial endospores. Contamination of Betadine with *Pseudomonas cepacia* has been reported.

Bromine is sometimes used in the form of gaseous methyl bromide to fumigate soil that will be used in the propagation of bedding plants. It is also used in some pools and indoor hot tubs because it does not give off the strong odor that chlorine does.

Chloramine, a combination of chlorine and ammonia, is less effective than other chlorine compounds at killing microbes, but superior at eliminating taste and odor problems. It is used in wound cleansing, root canal therapy, and is often added to water treatment procedures. But beware! Its residues will kill fish in aquaria and ponds. However, commercial products are available to neutralize this effect.

Alcohols

When mixed with water, alcohols denature protein. They are also lipid solvents and dissolve membranes. Ethyl and isopropyl alcohols can be used as skin antiseptics. Isopropyl alcohol is more often used because of legal regulation of ethyl alcohol. It disinfects skin where injections will be made or blood drawn. Alcohol disinfects but does not sterilize skin because it evaporates quickly and stays in contact with microbes for only a few seconds. It also does not penetrate deeply enough into pores in the skin. It kills vegetative microorganisms on the skin surface but does not kill endospores, resistant cells, or cells deep in skin pores. Ten to 15 minutes immersion in 70% ethyl alcohol is usually sufficient to disinfect a thermometer.

Phenols

Phenol and phenol derivatives called *phenolics* disrupt cell membranes, denature proteins, and inactivate enzymes. They are used to disinfect surfaces and to destroy discarded cultures because their action is not impaired by organic materials. Amphyl, which contains amylphenol, destroys vegetative forms of bacteria and fungi and inactivates viruses. It can be used on skin, medical instruments, dishes, and furniture. When used on surfaces, it retains its antimicrobial action for several days. The orthophenylphenol in Lysol gives it similar properties. A mixture of phenol derivatives called *cresols* is found in creosote, a substance used to prevent the rotting of wooden posts, fences, railroad ties, and such. However, because creosote is irritating to skin and is a carcinogen, its use is limited. The addition of halogens to phenolic molecules usually increases their effectiveness. Hexachlorophene and dichlorophene, which are halogenated phenols, inhibit staphylococci and fungi, respectively, on the skin and elsewhere. Chlorhexidine gluconate (Hibiclens), which is chlorinated and similar in structure to hexachlorophene, is effective against a wide variety of microbes even in the presence of organic material. It is a good agent for surgical scrubs.

PUBLIC HEALTH

Hexachlorophene

Hexachlorophene is an excellent skin disinfectant. In a 3% solution, it kills staphylococci and most other Gram-positive organisms, and its residue on skin is strongly bacteriostatic. Because staphylococcal skin infections can spread easily among newborn babies in hospitals, this antiseptic was used extensively in the 1960s for daily bathing of infants. The unforeseen price paid for controlling infections was permanent brain damage in infants bathed in it over a period of time. Hexachlorophene is absorbed through the skin and travels in the blood to the brain. Baby powder containing hexachlorophene killed 40 babies in France in 1972. Available in the United States today only by prescription, hexachlorophene is used routinely, though very cautiously, in hospital neonatal units because it is still the most effective agent for preventing the spread of staphylococcal infections.

TABLE 12.3

Properties of Chemical Antimicrobial Agents

Agent	Actions	Uses
Soaps and detergents	Lower surface tension, make microbes accessible to other agents	Hand washing, laundering, sanitizing kitchen and equipment
Surfactants	Dissolve lipids, disrupt membranes, denature proteins, and inactivate enzymes in high concentrations; act as wetting agents in low concentrations	Cationic detergents are used to sanitize utensils; an detergents to launder clothes and clean household objects; quaternary ammonium compounds are sometimes used as antiseptics on skin.
Acids	Lower pH and denature proteins	Food preservation
Alkalis	Raise pH and denature proteins	Found in soaps
Heavy metals	Denature proteins	Silver nitrate is used to prevent gonococcal infecti mercury compounds to disinfect skin and inanim objects, copper to inhibit algal growth, and selen inhibit fungal growth.
Halogens	Oxidize cell components in absence of organic matter	Chlorine is used to kill pathogens in water and to disinfect utensils; iodine compounds are used as antiseptics.
Alcohols	Denature proteins when mixed with water	Isopropyl alcohol is used to disinfect skin; ethylene and propylene glycol can be used in aerosols.
Phenols	Disrupt membranes, denature proteins, and inactivate enzymes; not impaired by organic matter	Phenol is used to disinfect surfaces and destroy dis cultures; amylphenol destroys vegetative organi and inactivates viruses on skin and inanimate ob chlorhexidine gluconate is especially effective as surgical scrub.
Oxidizing agents	Disrupt disulfide bonds	Hydrogen peroxide is used to clean puncture wour potassium permanganate to disinfect instrument
Alkylating agents	Disrupt structure of proteins and nucleic acids	Formaldehyde is used to inactivate viruses without destroying antigenic properties, glutaraldehyde sterilize equipment, betapropiolactone to destro hepatitis viruses, and ethylene oxide to sterilize inanimate objects that would be harmed by high temperatures.
Dyes	May interfere with replication or block cell wall synthesis	Acridine is used to clean wounds, crystal violet to treat some protozoan and fungal infections.

✓CHECKLIST

1. How does a surfactant act?
2. If bacteria can grow in soap, why do we use it to clean things?
3. Explain the antimicrobial actions of acridine, mercurochrome, and Lysol.
4. What are the drawbacks of using ethylene oxide?

PUBLIC HEALTH

Is Cleanliness Really All That Great?

As part of a class investigative project, a student found that while she did not use the disinfectant Trichlosan, it was present in her skin. She lived with someone who did use Trichlosan. Join us on the web site for this chapter to examine the debates about the use of Trichlosan. Do you want it on your cutting board?

PHYSICAL ANTIMICROBIAL AGENTS

For centuries, physical antimicrobial agen been used to preserve food. Ancient Egyptians dr ishable foods to preserve them. Scandinavians mac in the centers of pieces of dry, flat, crisp bread in (hang them in the air of their homes during the likewise they kept seed grains in a dry place. Oth both flour and grains would have molded during t and very moist winters. Europeans used heat in th canning process 50 years before Pasteur's work ex why heating prevented food from spoiling. Today cal agents that destroy microorganisms are still food preservation and preparation. Such agents r crucial weapon in the prevention of infectious Physical antimicrobial agents include various fo heat, refrigeration, desiccation (drying), irradiati filtration.

Figure 12.13 Preservation by drying. Sun drying is an ancient means of preventing the growth of microorganisms. These grapes will remain edible as raisins because microbes need more water than remains inside the dried fruit. (Link/Visuals Unlimited)

less palatable. It also allows bacteria to multiply while food is thawed, making the food more susceptible to bacterial degradation.

Freezing can be used to preserve microorganisms, but this requires a much lower temperature than that used for food preservation. Microorganisms are usually suspended in glycerol or protein to prevent the formation of large ice crystals (which could puncture cells), cooled with solid carbon dioxide (dry ice) to a temperature of −78°C, and then held there. Alternatively, they can be placed in liquid nitrogen and cooled to −180°C.

Drying

Drying can be used to preserve foods because the absence of water inhibits the action of enzymes. Many foods, including peas, beans, raisins, and other fruits, are often preserved by drying (**Figure 12.13**). Yeast used in baking also can be preserved by drying. Endospores present on such foods can survive drying, but they do not produce toxins. Dried pepperoni sausage and smoked fish retain enough moisture for microorganisms to grow. Because smoked fish is not cooked, eating it poses a risk of infection. Sealing such fish in plastic bags creates conditions that allow anaerobes such as *Clostridium botulinum* to grow.

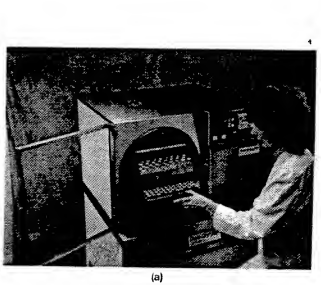
Drying also naturally minimizes the spread of infectious agents. Some bacteria, such as *Treponema pallidum*, which causes syphilis, are extremely sensitive to drying and die almost immediately on a dry surface; thus they can be prevented from spreading by keeping toilet seats and other bathroom fixtures dry. Drying of laundry in dryers or in the sunshine also destroys pathogens.

Freeze-Drying

Freeze-drying, or **lyophilization** (li-of″i-li-za′shun), is the drying of a material from the frozen state (**Figure 12.14**). This process is used in the manufacture of some brands of instant coffee; freeze-dried instant coffee has a more natural flavor than other kinds. Microbiologists use lyophilization for long-term preservation rather than for destruction of cultures of microorganisms. Organisms in vials are rapidly frozen in alcohol and dry ice or in liquid nitrogen, are then subjected to a high vacuum to remove all the water while in the frozen state, and finally are sealed under a vacuum. Rapid freezing allows only very tiny ice crystals to form in cells, so the organisms survive this process. Organisms so treated can be kept alive for years, stored under vacuum in the freeze-dried state.

RADIATION

Four general types of radiation—ultraviolet light, ionizing radiation, microwave radiation, and strong visible light (under certain circumstances)—can be used to control



(a)

(b)

...re 12.14 Freeze-drying ...philization) equipment.
...stoppering tray dryer, in which the trays ...the heat needed to remove moisture. ...completion of lyophilization (about 24 ...the device automatically stoppers the ...TS Systems, Inc., photo by Gary Gold ...graphy.) **(b)** A manifold dryer, in which ...se samples in vials of different sizes ...ttached via ports to the dryer, which ...s a vacuum to remove water. The ...will dry in 4 to 20 hours, depending ...ple thickness. Unlike the tray dryer, ...his allows samples to be added and ...d. (FTS Systems, Inc. photo by Gary ...tography.)

microorganisms and to preserve foods. Refer to the electromagnetic spectrum (◀Figure 3.4) to review their relative wavelengths and positions along the spectrum.

Ultraviolet Light

Ultraviolet (UV) light consists of light of wavelengths between 40 and 390 nm, but wavelengths in the 200-nm range are most effective in killing microorganisms by damaging DNA and proteins. Ultraviolet light is absorbed by the purine and pyrimidine bases of nucleic acids. Such absorption can permanently destroy these important molecules. Ultraviolet light is especially effective in inactivating viruses. However, it kills far fewer bacteria than one might expect because of DNA repair mechanisms. Once DNA is repaired, new molecules of RNA and protein can be synthesized to replace the damaged molecules (◀Chapter 7, p. 201). Lying in soil and exposed to sunlight for decades, endospores are resistant to UV damage because of a small protein that binds to their DNA. This changes the geometry of the DNA by untwisting it slightly, thereby making it resistant to the effects of UV irradiation.

Ultraviolet light is of limited use because it does not penetrate glass, cloth, paper, or most other materials, and it does not go around corners or under lab benches. It does penetrate air, effectively reducing the number of airborne microorganisms and killing them on surfaces in operating rooms and rooms that will contain caged animals (**Figure 12.15**). Ultraviolet lights lose effectiveness over time and should be monitored often. To help sanitize the air without irradiating humans, UV lights can be turned on when the rooms are not in use. Exposure to UV light can cause burns, as anyone who has had a sunburn knows, and can also damage the eyes; years of skin exposure can lead to skin cancer. Hanging laundry outdoors on sunny days takes advantage of the UV light pre sunlight. Although the quantity of UV rays in sur small, these rays may help kill bacteria on clothing cially diapers.

In some communities, UV light is replacing c in sewage treatment. When chlorine-treated sew fluent is discharged into streams or other bodies ter, carcinogenic compounds form and may ent food chain. The cost of removing chlorine befo charging treated effluent could add more than $1 year to the sewage bills of the average American and very few sewage plants do this. Running the s effluent under UV light before discharging it can c microorganisms without altering the odor, pH, or c cal composition of the water and without formir cinogenic compounds.

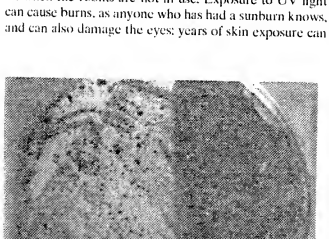


Figure 12.15 Ultraviolet radiation. The effects of UV exposure can be seen in this Petri plate of *Serratia marcescens*; the left side was exposed to UV rays while the right side was shielded. Most of the organisms on the left side have been killed. (Grant Heiman Photography)

Ionizing Radiation

X rays, which have wavelengths of 0.1 to 40 nm, and g rays, which have even shorter wavelengths, are forms *izing radiation*, so named because it can dislodge ele from atoms, creating ions. (Longer wavelengths are of *nonionizing radiation*.) These forms of radiation also kill microorganisms and viruses. Many bacteria are killed by absorbing 0.3 to 0.4 millirads of radiation; polioviruses are inactivated by absorbing 3.8 millirads. A **rad** is a unit of radiation energy absorbed per gram of tissue; a millirad is one-thousandth of a rad mans usually do not become ill from radiation unles are subjected to doses greater than 50 rads.

Irradiation is c by hospitals to sterilize food f immune-compr patients.

Ionizing radiation damages DNA and produce oxides, which act as powerful oxidizing agents in This radiation can also kill or cause mutations in h cells if it reaches them. It is used to sterilize plastic la tory and medical equipment and pharmaceutical ucts. It can be used to prevent spoilage in seafoo doses of 100 to 250 kilorads, in meats and poultry by doses of 50 to 100 kilorads, and in fruits by doses of 200 to 300 kilorads. (One kilorad equals 1,000 rads.) Many consumers in the United States reject irradiated foods for fear of receiving radiation, but such foods are quite safe—free of both pathogens and radiation. In Europe, milk and other foods are often irradiated to achieve sterility.

The bacterium Deinococcus ra durans, is rema able for its abi survive more th 1,000 times the amount of radia that would kill a human. It is bei studied as a can date for use in bioremediation sites contamina by radioactive materials

Microwave Radiation

Microwave radiation, in contrast with gamma, X-ray, and UV radiation, falls at the long-wavelength

laboratories. HEPA filters also capture organisms released in rooms occupied by patients with tuberculosis or in laboratories where especially dangerous microbes are studied, such as the maximum containment units shown in ◀Figure 15.14. These filters remove almost all organisms larger than 0.3 μm in diameter. Used filters are soaked in formalin before they are disposed of. Most rooms for patients with tuberculosis have an outer "hallway" outside the main door to the room. Negative air pressure inside the room should cause air from outside the room to be sucked into it whenever the door is opened. However, tests have shown that some air still does escape from the room; hence the need for the little containment "hallway" outside. Just remember to always put on your mask *before* you enter the little "hallway," as it will have some TB germs in it!

OSMOTIC PRESSURE

High concentrations of salt, sugar, or other substances create a hyperosmotic medium, which draws water from microorganisms by osmosis (◀Chapter 4, p. 108). **Plasmolysis** (plaz-mol'i-sis), or loss of water, severely interferes with cell function and eventually leads to cell death. The use of sugar in jellies, jams, and syrups or salt solutions in curing meat and making pickles plasmolyzes most organisms present and prevents growth of new organisms. A few halophilic organisms, however, thrive in these conditions and cause spoilage, especially of pickles, and some fungi can live on the surface of jams.

Properties of physical antimicrobial agents are summarized in **Table 12.5**.

✓CHECKLIST

1. Filtering water through paper does not sterilize the water. How, then, can membrane filters sterilize water?
2. Can a home microwave oven be used to sterilize items? Why or why not?
3. Are ice cubes safe sources of water in areas with poor water supplies?
4. Are pasteurized products sterilized?

TABLE 12.5

Properties of Physical Antimicrobial Agents

Agent	Action	Use
Dry heat	Denatures proteins	Oven heat used to sterilize glassware and metal objects; open flame used to incinerate microorganisms.
Moist heat	Denatures proteins	Autoclaving sterilizes media, bandages, and many kinds of hospital and laboratory equipment not damaged by heat and moisture; pressure cooking sterilizes canned foods.
Pasteurization	Denatures proteins	Kills pathogens in milk, dairy products, and beer.
Refrigeration	Slows the rate of enzyme-controlled reactions	Used to keep fresh foods for a few days; does not kill most microorganisms.
Freezing	Greatly slows the rate of most enzyme-controlled reactions	Used to keep fresh foods for several months; does not kill microorganisms; used with glycerol to preserve microorganisms.
Drying	Inhibits enzymes	Used to preserve some fruits and vegetables; sometimes used with smoke to preserve sausages and fish.
Freeze-drying	Dehydration inhibits enzymes	Used to manufacture some instant coffees; used to preserve microorganisms for years.
Ultraviolet light	Denatures proteins and nucleic acids	Used to reduce the number of microorganisms in air in operating rooms, animal rooms, and where cultures are transferred.
Ionizing radiation	Denatures proteins and nucleic acids	Used to sterilize plastics and pharmaceutical products and to preserve foods.
Microwave radiation	Absorbs water molecules, then releases microwave energy to surroundings as heat	Cannot be used reliably to destroy microbes except in special media-sterilizing equipment.
Strong visible light	Oxidation of light-sensitive materials	Can be used with dyes to destroy bacteria and viruses; may help sanitize clothing.
Sonic and ultrasonic waves	Cause cavitation	Not a practical means of killing microorganisms but useful in fractionating and studying cell components.
Filtration membranes	Mechanically removes microbes	Used to sterilize media, pharmaceutical products, and vitamins, in manufacturing vaccines, and in sampling microbes in air and wate
Osmotic pressure	Removes water from microbes	Used to prevent spoilage of foods such as pickles and jellies.